Nitric oxide mediated venodilator effects of nebivolol

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- 1 Nebivolol, a selective β_1 -adrenoceptor antagonist with antihypertensive effects, has haemodynamic effects suggestive of a direct vasodilator action.
- 2 The dorsal hand vein technique was used to determine whether nebivolol has venodilator action *in vivo* in man.
- 3 Nebivolol and atenolol were infused into the phenylephrine precontracted superficial hand veins of 11 healthy male volunteers. In separate studies L-NMMA ($0.1 \mu\text{g min}^{-1}$) was pre- and co-infused with nebivolol to determine whether nitric oxide (NO) mediated mechanisms were present. Further studies with prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) precontraction were performed to exclude an α -adrenergic antagonistic effect of nebivolol. Effects of L-NMMA infusion on nitroglycerin venodilation were also determined.
- 4 Nebivolol produced a dose dependent venodilation, ($72 \pm 18\%$ maximum), whereas atenolol produced no significant venodilation. At doses of nebivolol producing plasma concentrations comparable with plasma levels achieved after standard oral dosing (10^{-13} – 10^{-12} mol min^{-1}) small ($14 \pm 6\%$ and $23 \pm 8\%$) but significant ($P < 0.05$) venodilation was observed.
- 5 The venodilator response to nebivolol was significantly reduced by infusion of L-NMMA (maximum dilation 18% vs 72% , $P < 0.01$). Venodilator responses to nitroglycerin were unaffected by L-NMMA infusion. A venodilator effect to nebivolol was also seen following precontraction with $\text{PgF}_{2\alpha}$ ($40 \pm 20\%$ maximum).
- 6 Nebivolol has nitric oxide mediated, venodilator effects in man.

Keywords nebivolol β -adrenoceptor blocker veins endothelium nitric oxide

Introduction

Nebivolol, a racemic drug, is a selective β_1 -adrenoceptor antagonist, of which the (+)-isomer is one hundred times more potent a β_1 -adrenoceptor antagonist than the (–)-isomer [1]. Nebivolol maintains cardiac output by increasing stroke volume, whilst decreasing systemic vascular resistance in human volunteers and in animal studies [1]. This effect on peripheral vascular resistance does not appear to be due to effects on α -adrenoceptors, potassium or calcium channel blockade, peripheral sympathetic nervous system activity or by direct action on endothelial cells at low concentrations [2, 3]. Some work suggests that the vasodilatory effects are mediated through nitric oxide. Nebivolol induces an endothelial dependent relaxation of canine coronary arteries and potentiates ADP induced endothelial dependent relaxation via

endothelial dependent relaxation factor (EDRF), now known to be nitric oxide [5].

The aims of the present study were to determine whether nebivolol has venodilator properties in man and whether such effect is mediated through nitric oxide.

Methods

Subjects

Eleven healthy male volunteers, mean age 30 years (range 26–36 years) were studied. All had a normal history, physical examination, full blood count, urea

and electrolytes, liver function tests, random blood glucose, urinalysis and resting ECG. Mean height was 180 cm (range 173–189 cm) and mean weight 82 kg (range 72–107 kg). Mean blood pressure was 123/78 (range 101–145/64–90) mm Hg. Subjects refrained from caffeine for 24 h prior to each study. Written informed consent was obtained from all subjects and studies were approved by the Newcastle Joint Ethics Committee.

Dorsal hand vein technique

The dorsal hand vein technique as modified by Aellig [6] and previously described in detail was used [7]. Studies were performed in a temperature controlled ($23 \pm 2^\circ \text{C}$) laboratory. Subjects lay semi-supine with the study arm resting at a 30° slope to ensure venous drainage. A 23 gauge butterfly needle was inserted into a suitable vein, on the dorsum of the hand and a continuous infusion of 0.9% saline at 0.3 ml min^{-1} commenced. A linear variable differential transducer (LVDT, Schaevitz Engineering) was then placed over the study vein 10 mm downstream from the needle tip. The position of the core, linearly related to voltage output was recorded on a strip chart recorder. Readings of the position of the core were made before and during inflation of a sphygmomanometer cuff on the same arm to 40 mm Hg to produce venous filling. The baseline venodilation during saline infusion with the cuff inflated was defined as 100% relaxation, the recording with the cuff not inflated was defined as 100% constriction. Venodilator responses to nebigolol, atenolol or nitroglycerin following precontraction with phenylephrine or $\text{PGF}_{2\alpha}$ was defined as a percentage of the baseline obtained during initial saline infusion by the formula $[(V-X)/(X-Y)] \times 100$ where V is vein diameter with vasodilator, X is baseline (saline) vein diameter and Y is constricted (phenylephrine pre vasodilator) vein diameter. Blood pressure and heart rate were monitored at 10 min intervals on the opposite arm. All drug solutions were infused at 0.3 ml min^{-1} .

Studies

Studies were separated by not less than 1 week.

Nebivolol effects on phenylephrine precontracted hand veins

The action of nebigolol on precontracted hand veins was determined in eight subjects. Saline was infused for 30 min followed by incremental doses of phenylephrine (range 150–1500 ng min^{-1}) until the vein was constricted to 70–80% of baseline. The diluent carrier solution and then nebigolol (MW 442) at increasing doses ($1\text{--}10,000 \text{ ng min}^{-1}$, 2×10^{-12} to $2 \times 10^{-8} \text{ mol min}^{-1}$) were infused concomitantly with the phenylephrine constrictor dose. All responses were recorded after at least 7 min infusion time. Dilator responses to nebigolol were observed to have plateaued at 7 min as responses recorded at 10 min in initial studies were not significantly different.

L-NMMA studies

The effect of L-NMMA on the vasodilator effect of nebigolol was determined in seven of eight subjects studied in the first phenylephrine studies. One subject who had no venodilator response to nebigolol was excluded. Saline was infused for 30 min followed by phenylephrine until the vein was constricted to 70–80% of baseline. L-NMMA was then infused concomitantly at a dose of $0.1 \mu\text{g min}^{-1}$ for 30 min and then during increasing nebigolol doses (2×10^{-12} to $2 \times 10^{-8} \text{ mol min}^{-1}$). Control studies of L-NMMA effects in nitroglycerin venodilation were performed in four subjects. Following phenylephrine precontraction nitroglycerin was infused at doses of $1\text{--}100 \text{ pg min}^{-1}$. L-NMMA was then infused for 30 min, during which time baseline precontraction returned and responses to nitroglycerin were again determined.

Atenolol effects on phenylephrine precontracted hand veins

A 30 min saline infusion was followed by incremental doses of phenylephrine until the study vein was constricted to 70–80% of baseline. Increasing doses of atenolol (MW 266) were infused ($0.5\text{--}5,000 \text{ ng min}^{-1}$, 2×10^{-12} to $2 \times 10^{-8} \text{ mol min}^{-1}$) concomitantly with the phenylephrine constrictor dose. The response to each atenolol dose was recorded after 7 min infusion.

Lower dose and duration of effect of nebigolol

To assess the response of human hand veins to lower doses of nebigolol and to characterise the duration of venodilator effects, further studies were performed in nine subjects. Following 30 min infusion of saline, a phenylephrine precontraction dose was established, as described above. Nebigolol was then infused incrementally (2×10^{-13} to $2 \times 10^{-9} \text{ mol min}^{-1}$) with the concomitant infusion of the phenylephrine precontraction dose. When a maximum dilation at $2 \times 10^{-9} \text{ mol min}^{-1}$ was reached, the nebigolol infusion was stopped and the precontraction dose of phenylephrine was infused for a further 60 min. Hand vein dilation was measured every 10 min.

Nebivolol effect on $\text{PGF}_{2\alpha}$ precontracted hand veins

To exclude α -adrenergic blockade as the cause for the nebigolol induced venodilation of phenylephrine constricted veins, the effects of nebigolol in $\text{PGF}_{2\alpha}$ constricted veins were determined, in nine subjects. Following 30 min infusion of saline, a precontraction dose of $\text{PGF}_{2\alpha}$ that produced 70–80% constriction of baseline distension was established and nebigolol was then infused concomitantly (2×10^{-12} to $2 \times 10^{-8} \text{ mol min}^{-1}$).

Statistical analysis

Statistical analysis was performed using Student's *t*-test for paired data, *P* values < 0.05 were con-

sidered significant. Dose-response curves to nebivolol and atenolol were analysed by repeated measures ANOVA.

Results

Action of nebivolol on phenylephrine precontracted hand veins

The mean precontraction dose of phenylephrine infused was 602 ± 598 ng min⁻¹ (mean $\pm$ s.d., range 75–1500 ng min⁻¹). The degree of precontraction was $79 \pm 14\%$ expressed relative to the baseline distension during saline infusion. The diluent did not significantly affect the phenylephrine precontraction (mean change $2 \pm 7\%$, $P = 0.44$). A dose-dependent venodilator response to nebivolol was seen in seven of eight subjects (Figure 1). Maximum venodilation was $72 \pm 18\%$. In three subjects, a biphasic response was seen with the maximum venodilator response occurring at doses lower than the highest infused dose of nebivolol used.

L-NMMA effects

Nebivolol induced venodilation was significantly reduced following L-NMMA infusion, at all doses of nebivolol infused, with a maximum dilation of $18 \pm 19\%$ (Figure 1). Mean phenylephrine precontraction dose was 893 ± 602 (range 150–1500 ng min⁻¹) which was not significantly different from the initial

nebivolol study ($P = 0.19$). The degree of precontraction was $79 \pm 5\%$. L-NMMA infused alone in phenylephrine constricted hand veins did not affect the degree of precontraction (72 ± 19 vs $74 \pm 15\%$, $P = 0.77$). Nitroglycerin induced venodilation was unaltered by L-NMMA; mean venous dilation before/following L-NMMA in four subjects (1 pg min⁻¹ 17 vs 20% , 10 pg min⁻¹ 49 vs 42% , 100 pg min⁻¹ 61 vs 71%).

Atenolol action on phenylephrine precontracted hand veins

Mean phenylephrine precontraction dose was 361 ± 238 (range 19–600) ng min⁻¹. The degree of precontraction was $85 \pm 6\%$ which was not significantly different from the degree of precontraction obtained in studies with nebivolol ($P = 0.19$). There was no significant venodilation at any dose of atenolol (Figure 2).

Lower dose and duration of nebivolol action

Significant venodilation was observed at all doses of nebivolol (Figure 3a). Small but significant venodilation occurred (mean $\pm$ s.d. dilation compared with baseline; $14 \pm 6\%$ at 2×10^{-13} , $P < 0.05$; $23 \pm 8\%$ at 2×10^{-13} mol min⁻¹, $P < 0.05$) at infusions equivalent to the plasma concentrations achieved with standard oral dosing for hypertension and angina (0.1 to 10 ng ml⁻¹) assuming blood flow of 1 ml min⁻¹ through a single hand vein. There was no significant difference between the mean dilation ($51 \pm 17\%$) at nebivolol

Figure 1 Venodilator effects of nebivolol in phenylephrine constricted hand veins of healthy volunteers without (Δ , $n = 8$) and during L-NMMA infusion ($\square$, $n = 7$). Data are mean $\pm$ s.e. mean.

Figure 2 Venodilator effects of nebivolol (Δ) and atenolol ($\square$) in phenylephrine constricted hand veins. Data are mean $\pm$ s.e. mean ($n = 9$).

dose 2×10^{-9} mol min⁻¹ obtained in the low dose nebigolol studies (Figure 1) and the mean dilation of ($33\% \pm 10\%$) seen at nebigolol dose 2.3×10^{-9} mol min⁻¹ obtained in the nebigolol studies conducted with L-NMMA ($P = 0.39$) (Figure 3a). Persistence of

significant venodilator effect was seen beyond 60 min, however, there was a noticeable decrease in the degree of dilation in most individuals after 30 min (Figure 3b). The degree of precontraction was $84 \pm 6\%$ with a phenylephrine precontraction dose of 341 ± 122 (range 45–1250) ng min⁻¹. There was no significant difference in either the dose of phenylephrine ($P = 0.45$) or the degree of precontraction ($P = 0.24$) compared with the previous nebigolol studies.

Nebivolol effect on PGF_{2α} constricted hand veins

Mean PGF_{2α} constriction dose was 960 ± 400 (range 18–3750) ng min⁻¹. The degree of precontraction was $74 \pm 11\%$. This was not significantly different from the mean precontraction of the first study where phenylephrine was the constrictor ($P = 0.74$). A statistically significant nebigolol induced venodilation was seen at all doses of nebigolol infused (Figure 4). There was no significant difference in the degree of nebigolol induced venodilation with either phenylephrine or PGF_{2α} as the precontractor ($P = 0.74$). A biphasic response was observed in some subjects as seen in the phenylephrine studies.

Discussion

These results confirm that nebigolol has a venodilator action in human hand veins and that this effect is not shared by atenolol. The inhibitory effect of L-NMMA indicates that venodilation due to nebigolol is primarily mediated through nitric oxide. This nitric oxide mediated, endothelial dependent venodilator effect described has not previously been described in human

Figure 3 a) Venodilator effects of nebigolol at lower dose infusions on phenylephrine constricted hand veins and b) Duration of nebigolol venodilator effect during 60 min following discontinuation of 2×10^{-9} mol min⁻¹ nebigolol infusion. Data are mean $\pm$ s.e. mean ($n = 9$).

Figure 4 Venodilator effect of nebigolol with PGF_{2α} as venoconstrictor. Data are mean $\pm$ s.e. mean ($n = 8$).

vasculature with other β -adrenoceptor blocking drugs. The observations that atenolol has no significant venodilator effect in human hand veins, indicate that nitric oxide mediated venodilation is not a class effect of all β -adrenoceptor antagonists and may be unique to nebivolol. Studies of other vasodilatory β -adrenoceptor antagonists using the hand vein model and L-NMMA would be of interest.

L-NMMA infusion did not modify the venoconstrictor action of phenylephrine, an observation in keeping with previous observations that L-NMMA does not alter vasoconstrictor effect of noradrenaline [8]. This and the lack of effect of L-NMMA on venodilator responses to nitroglycerin excludes a non-specific venoconstrictor effect of L-NMMA and suggests that nebivolol is acting through endogenous nitric oxide. The venodilator responses with $\text{PGF}_{2\alpha}$ as the venoconstrictor exclude an α -adrenoceptor antagonist action of nebivolol as the cause of the venodilation observed in the phenylephrine studies. This is in keeping with previous work which indicates that nebivolol has no effects at the α -adrenoceptor [2, 3]. Some animal work has found that β -adrenoceptor mediated vascular relaxation is mediated via nitric oxide release from the endothelium, although this has not been a consistent finding and appears to be specific to certain species and vascular beds [9–11]. Nebivolol has a relatively weak affinity for β_2 -compared with β_1 -adrenoceptors, and one possible explanation for our findings is that nebivolol is acting as a partial agonist at vascular β_2 -adrenoceptors which mediate venodilation via nitric oxide. Racemic nebivolol lacks intrinsic sympathomimetic activity in isolated right atria of reserpinised rats and in reserpinised anaesthetised dogs, suggesting that such an action is unlikely [2]. Also evidence that β -adrenoceptor mediated vasodilatation in human hand veins is mediated via nitric oxide is lacking. Alternatively nebivolol may be acting through a non β -adrenoceptor mediated mechanism. It would be important to investigate the mechanism underlying this effect as it could lead to the development of selective modulators of nitric oxide synthase. Further studies examining the vasodilatory effects of individual nebivolol isomers and the effect of coinfusion of propranolol on nebivolol induced venodilation would be of interest.

In three of seven subjects a biphasic venodilator of nebivolol on phenylephrine constricted veins was recorded. This may be due to tachyphylaxis but is perhaps more likely to be a non-specific venoconstrictor effect of the higher doses infused. Non-specific venodilator effects to the preservative sodium metabisulphite have been previously described [7]. The results of the study using nebivolol at lower doses show that venodilation occurs at infusions equivalent to the plasma concentrations achieved with standard oral dosing for hypertension and angina (0.1 to 10 ng ml^{-1}) suggesting that a vasodilatory effect through nitric oxide may account for the haemodynamic profile of nebivolol when administered in these doses. The persistence of venodilator action was seen to occur beyond 60 min in some individuals, however, there was a marked decrease in venodilation in most subjects after 30 min. The nature of this prolonged effect

is unclear however, it is possibly a result of persistent tissue binding of nebivolol but may represent a sustained effect on vascular nitric oxide synthase.

Other β -adrenoceptor antagonists have vasodilator effects, although the modes of action of most of these are not thought to be endothelial dependent, and those that have been shown to act on the endothelium have not been shown to act via nitric oxide. Janczewski *et al.* [12] suggest that carteolol facilitates the abluminal release of nitric oxide caused by α_2 -adrenergic activation and causes the intraluminal release of vasodilator prostaglandin in animal *in vitro* studies but studies on human vasculature have not been performed. Vasodilation by the β -adrenoceptor blockers, such as labetalol, timolol and propranolol, have been shown to be mediated via their weak action on α -adrenoceptors [13]. Nipradilol is another non-selective β -adrenoceptor blocker with vasodilator activities which are thought to be due to its weak α -adrenoceptor blocking actions [14]. However, it may also have a nitroglycerin like action, as it contains nitroxy residues in its molecule [15]. A recent study showed that in rat arterioles and venules it appeared to have a dilator effect when applied topically, which did not occur with propranolol, atenolol and labetalol. The study did not however, use endothelial denuded tissue or nitric oxide synthase inhibitors and hence an endothelial dependent venodilation has not been established [16]. Carvedilol has been shown to have vasodilator activity mediated primarily via α_1 -adrenergic blockade [17]. However, a more recent study [18] suggests that an additional, as yet undefined, mechanism could be involved.

β -adrenoceptor blocking drugs such as xamoterol, have in the past been used in the treatment of congestive heart failure. Most of these drugs have a significant negative inotropic effect which severely restricts their use. Recently there has been renewed interest in this form of therapy [19]. The nitric oxide mediated venodilation induced by nebivolol, may decrease preload and therefore be of benefit in the treatment of heart failure, particularly, when it is this action that is thought to be responsible for the maintenance of cardiac output found with nebivolol. Further clinical trials investigating the haemodynamic effects on nebivolol in animal and clinical studies would clarify this.

Endothelial dysfunction is well described in the peripheral vasculature of patients with hypertension and in the coronary arteries of patients with angina [20–23]. This may be a pathophysiological factor in coronary and cerebral vessel thrombosis. Further evidence is emerging that interference with the L-arginine/nitric oxide synthesis pathway is important in the aetiology of hypertension and that correction of nitric oxide levels, either directly or by addition of L-arginine, results in lowering of the blood pressure [24–27]. Enhancing vascular endothelial response in these situations could result in nebivolol having greater clinical benefits in terms of stroke and myocardial infarction than other β -adrenoceptor blockers.

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